Preliminary PD data suggest that MGCD0103 can inhibit HDAC activity in a dose-dependent manner, and induce histone acetylation in peripheral blood mononuclear cells. Both these effects have been shown to persist for 72 hours following dosing.

301 POSTER

The design, synthesis and biological evaluation of a set of C2-aryl substituted pyrrolo[2,1-c][1,4]benzodiazepine dimers

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The pyrrolobenzodiazepines (PBDs) are a family of naturally occurring antitumour antibiotics. Many of the most potent family members contain an *endo-exo* unsaturated motif associated with the pyrrolo C-ring of the PBD.

We have previously reported the synthesis and potent *in vitro* and *in vivo* antitumour activity of SG2285, a PBD dimer which retains the *endo-exo* unsaturation motif in the form of a C2-aryl substituent conjugated to a 2,3 double bond. We now report the synthesis and biological evaluation of a set of 10 analogs with diverse C2-aryl substituents. These C2-aryl substituents were selected on the basis of the biological evaluation of approximately 80 C2-aryl PBD monomers.

The novel dimers were prepared from a key enol triflate PBD intermediate by Pd(0) catalysed coupling with the appropriate aryl boronic acids. Removal of the N10 Troc protecting group afforded the free PBD imines, which were converted to their bisulphite adducts in order to improve water solubility and modulate the DNA-reactivity of the PBD unit.

The C2-aryl PBD dimers were evaluated in the K562 human leukaemia cell line (96 hrs continuous exposure) with IC_{50} values in the range 0.02−43 nM. The 3-methoxyphenyl analog (SG2965) and the 3,4-benzodioxole analogs (SG2962 and 2965) were particularly potent with IC_{50} values of 20, 80 and 50 pM, respectively. These molecules were also found to be efficient DNA interstrand cross-linking agents in both plasmid and cellular DNA. In the case of the bisulphite adducts, the cross-links were found to form more slowly in cells reaching a maximum at approximately 24 hrs.

These studies confirm the potent activity of C2-aryl PBD dimers of this type, and SG2285 is currently undergoing preclinical development.

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Processing of 1-nitroacridine-induced DNA-DNA cross-links by topoisomerase I is associated with enhanced cellular survival: a possible role of topoisomerase I in the removal of DNA cross-links

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1-nitroacridines like nitracrine (Ledakrin) are potent antitumor agents with clinical activity toward ovary, lung and breast cancers. These compounds are activated by bioreduction and bind covalently to DNA. This is accompanied by formation of DNA cross-links and covalent DNA-protein complexes. Earlier studies showed that 1-nitroacridines could be activated by thiols *in vitro* to reactive species which bind DNA and proteins. On the other hand, DNA topoisomerases have been indicated in the processing of DNA adducts. We here show that modification of plasmid DNA with nitracrine and, to a lesser degree, the nitracrine derivative C-857 inhibited the catalytic activity of purified topoisomerase I in a dose-dependent manner. The inhibition is associated with the induction of DNA single strand breaks and the formation of covalent DNA-topoisomerase I complexes. In contrast, no detectable effects were observed for purified human topoisomerase IIα. Further studies revealed that both nitracrine and C-857 form unusual DNA cross-links between different DNA molecules. Unexpectedly, the high molecular weight cross-linked DNA formed in the presence of nitracrine, but not by C-857, was completely resolved after further incubation with topoisomerase I. Accordingly, the In Vivo link assay revealed the formation of covalent DNA-topoisomerase I complexes in LNCaP cells after treatment with nitracrine but not with C-857. DNA cross-linking was accompanied by the formation of double stranded DNA breaks that were particularly pronounced for cells treated by C-857 suggesting that the topoisomerase I-mediated processing of the cross-linked DNA may play a role in DNA repair. In agreement, cells with decreased topoisomerase I levels showed increased sensitivity to nitracrine but unchanged sensitivity to C-857. In conclusion, our results suggest a novel role for topoisomerase I in the removal of DNA-DNA cross-links which is accompanied by increased cellular survival.

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Additive and synergistic effects of irofulven and capecitabine in human prostate cancer cells

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Background: Irofulven (6-hydroxymethylacylfulvene, MGI 114) is a novel DNA-interacting anticancer drug derived from the mushroom natural product, illudin S. Irofulven displays a broad range of activity against human tumors in vitro and in vivo and is currently under study in clinical trials as a single agent and in combination with several other anticancer drugs. To optimize the clinical use of irofulven, the present study examined the cytotoxic effects of combining irofulven with 5′-DFUR or 5-FU, the active metabolite of capecitabine, in human prostate cancer cells.

Materials and Methods: Antiproliferative effects of irofulven, 5′-DFUR and 5-FU were evaluated in two prostate cancer cell lines, PC3 and DU145, with different expression levels of thymidine phosphorylase (TP), a key enzyme for capecitabine metabolism. Drug interaction studies were performed using isobolograms according to the method of Chou & Talalay.

Results: Single agent irofulven produced cytotoxic effects against human PC3 and DU145 prostate cancer cells with IC50s of 4.2+0.9 μM and 1.4+0.6 μM, respectively. Sensitivity to 5′-DFUR was directly correlated with TP expression level. PC3 cells expressed less TP and were less sensitive to 5′-DFUR than DU145 (IC50s of 62 and 33 μM, respectively). Combination of irofulven with 5′-DFUR produced additive/synergistic activity over a broad range of concentrations in both PC3 and DU145 cells, and similar effects were observed when irofulven was combined with 5-FU. While there was no clear schedule dependency for the tested combinations, both cell lines showed a trend favoring 5-FU/5′-DFUR exposure prior to irofulven. Cell cycle analysis showed consistent outcomes for 5′-DFUR and 5-FU with accumulation of cells in the S-phase of cell cycle, while combinations with irofulven were associated with cell cycle blockage in G1/S. Irofulven induced cellular apoptosis as a single agent, however, in combination with either 5′-DFUR or 5-FU the observed apoptosis was markedly increased.

Conclusion: Irofulven displays additive/synergistic anti-proliferative effects when combined with 5′-DFUR and 5-FU over a broad range of concentrations in human prostate cancer cells. Cell cycle arrest in S-phase and apoptosis appear as primary mechanisms of cytotoxicity of irofulven-based combinations. Based on these data, the irofulven-capecitabine combination should be further explored using a schedule that preferably gives capecitabine prior to irofulven.

304 POSTER

Combination treatment of new molecular-targeted therapies and the DNA minor groove binder brostallicin

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Brostallicin is a DNA minor groove binder, currently in Phase I/II trials as single agent or in combination. It has antitumor effect and strong pro-apoptotic activity in experimental tumors. The mechanism of DNA interaction is novel, since it binds covalently to DNA only in the presence of glutathione and glutathione S-transferase (GSH/GST). As a consequence, brostallicin is more effective on tumors expressing relatively high levels of GSH/GST. Multiple combinations of brostallicin with “classical” antitumor drugs have been previously studied; synergy was observed with cisplatin, gemcitabine and irinotecan. Based on the role of combination chemotherapy in cancer treatment, and its importance for the efficacy of newer therapies, we evaluated whether brostallicin could synergize with novel molecular-targeted dugs. In this study we examined the effects of combining brostallicin and different molecular-targeted agents (such as erlotinib or gefitinib or imatinib) on different experimental tumors. In vitro and in vivo studies were performed on tumors cells sensitive to brostallicin; for each combination, tumor cells sensitive to the kinase inhibitor were selected. In vitro cells were treated with increasing doses of brostallicin and/or the kinase inhibitor for 72 h. At the end of treatment, cell proliferation of treated and control cells was determined by a cellular ATP monitoring system. In vivo, DU145 human prostate carcinoma, A549 human lung carcinoma, HCT-116 human colon carcinoma and K562 human AML models, transplanted in nude mice were used to determine the effect of brostallicin/molecular targeted agents combinations. Drugs were administered at their best schedule and route and the simultaneous treatment was used for all the tested combinations. The effect of the antitumor treatment was determined as the delay, in days, in the onset